

WHAT IS CLAIMED IS:

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1. A method for detecting a single nucleotide polymorphism in a target comprising:
 - a) hybridizing a detector primer and a second primer to the target such that extension of the second primer by polymerase displaces the detector primer from the target sequence, wherein the detector primer comprises a diagnostic nucleotide for the single nucleotide polymorphism which is a 3' terminal nucleotide of the detector primer or about one to four nucleotides from the 3' terminal nucleotide;
 - b) extending the detector primer and the second primer with polymerase to produce a displaced detector primer extension product;
 - c) determining an efficiency of detector primer extension, and;
 - d) detecting the presence or absence of the single nucleotide polymorphism based on the efficiency of detector primer extension.
 2. The method of Claim 1 wherein the single nucleotide polymorphism is identified using the detector primer.
 3. The method of Claim 2 wherein the single nucleotide polymorphism is identified using multiple detector primers, each comprising a different diagnostic nucleotide.
 4. The method of Claim 3 wherein two detector primers are used to identify which of two possible alleles is present in the target sequence.
 5. The method of Claim 3 wherein four detector primers are used to identify the nucleotide present in the target sequence at the position of the single nucleotide polymorphism.
 6. The method of Claim 3 wherein each of the multiple detector primers has a different 5' tail sequence.
 7. The method of Claim 1 wherein the detector primer further comprises a nucleotide which forms a nondiagnostic mismatch with the target sequence.
 8. The method of Claim 7 wherein the nondiagnostic nucleotide is positioned within fifteen nucleotides of the diagnostic nucleotide in the detector primer.

9. The method of Claim 8 wherein the nondiagnostic nucleotide is positioned 1-5 nucleotides from the diagnostic nucleotide in the detector primer.

10. The method of Claim 9 wherein the nondiagnostic nucleotide is adjacent to the diagnostic nucleotide in the detector primer.

11. The method of Claim 7 wherein the detector primer is about 15-36 nucleotides long.

12. The method of Claim 11 wherein the detector primer is about 18-24 nucleotides long.

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~~13. The method of Claim 1 wherein the second primer is an amplification primer.~~

14. The method of Claim 13 wherein the amplification reaction is selected from the group consisting of SDA, 3SR, NASBA, TMA and PCR.

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15. The method of Claim 1 wherein the detector primer is about 12-50 nucleotides long.

16. The method of Claim 15 wherein the detector primer is about 12-24 nucleotides long.

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17. The method of Claim 16 wherein the detector primer is about 12-19 nucleotides long.

~~18. The method of Claim 1 wherein the presence or absence of the single nucleotide polymorphism is detected by means of a label associated with the detector primer.~~

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19. The method of Claim 18 wherein the label becomes detectable upon extension of the detector primer or produces a change in signal upon extension of the detector primer.

20. The method of Claim 19 wherein the label is a fluorescent donor/quencher dye pair and a decrease in donor dye fluorescence is detected as an indication of the presence of the single nucleotide polymorphism.

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21. The method of Claim 19 wherein a change in fluorescence polarization is detected as an indication of the presence of the single nucleotide polymorphism.

22. The method of Claim 1 wherein a single nucleotide polymorphism in an HFE gene is detected.

23. The method of Claim 22 wherein the single nucleotide polymorphism is detected in exon 4 or exon 2 of the HFE gene.

24. The method of Claim 1 wherein the efficiency of detector primer extension is determined
5 quantitatively.

25. A method for detecting a single nucleotide polymorphism in a target comprising, in an isothermal nucleic acid amplification reaction:

- 10 a) hybridizing a detector primer to the target, wherein the detector primer comprises a diagnostic nucleotide for the single nucleotide polymorphism about one to four nucleotides from a 3' terminal nucleotide of the detector primer which is complementary to the target sequence;
- b) amplifying the target by hybridization and extension of the detector primer;
- c) determining an efficiency of detector primer extension, and;
- 15 d) detecting the presence or absence of the single nucleotide polymorphism based on the efficiency of detector primer extension.

26. The method of Claim 25 wherein the single nucleotide polymorphism is identified using the detector primer.

27. The method of Claim 26 wherein the single nucleotide polymorphism is identified using two or more detector primers, each comprising a different diagnostic nucleotide.

28. The method of Claim 27 wherein two detector primers are used to identify which of two possible alleles is present in the target sequence.

29. The method of Claim 27 wherein four detector primers are used to identify the nucleotide present in the target sequence at the position of the single nucleotide polymorphism.

30. The method of Claim 27 wherein each of the multiple detector primers has a different 5' tail sequence.

31. The method of Claim 25 wherein the detector primer further comprises a nucleotide which forms a nondiagnostic mismatch with the target sequence.

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32. The method of Claim 31 wherein the nondiagnostic nucleotide is positioned within fifteen nucleotides of the diagnostic nucleotide in the detector primer.

5 33. The method of Claim 32 wherein the nondiagnostic nucleotide is positioned 1-5 nucleotides from the diagnostic nucleotide in the detector primer.

34. The method of Claim 33 wherein the nondiagnostic nucleotide is adjacent to the diagnostic nucleotide in the detector primer.

10 35. The method of Claim 31 wherein the detector primer is about 15-36 nucleotides long.

36. The method of Claim 35 wherein the detector primer is about 18-24 nucleotides long.

15 37. The method of Claim 25 wherein the isothermal amplification reaction is selected from the group consisting of SDA, 3SR, NASBA and TMA.

38. The method of Claim 25 wherein the detector primer is about 12-50 nucleotides long.

20 39. The method of Claim 38 wherein the detector primer is about 12-24 nucleotides long.

40. The method of Claim 39 wherein the detector primer is about 12-19 nucleotides long.

25 41. The method of Claim 25 wherein the presence or absence of the single nucleotide polymorphism is detected by means of a label associated with the detector primer.

42. The method of Claim 41 wherein the label becomes detectable upon extension of the detector primer or produces a change in signal upon extension of the detector primer.

30 43. The method of Claim 42 wherein the label is a fluorescent donor/quencher dye pair and a decrease in donor dye fluorescence is detected as an indication of the presence of the single nucleotide polymorphism.

35 44. The method of Claim 42 wherein a change in fluorescence polarization is detected as an indication of the presence of the single nucleotide polymorphism.

45. The method of Claim 25 wherein the efficiency of detector primer extension is determined quantitatively.

46. An oligonucleotide which comprises:

- 5 a) a nucleotide sequence which hybridizes to an internal segment of a target nucleic acid downstream from a hybridization site for a primer such that extension of the primer displaces the oligonucleotide from the target sequence, and;
- 10 b) a 3' terminal nucleotide or a nucleotide about one to four nucleotides from the 3' terminal nucleotide which is diagnostic for a single nucleotide polymorphism which may be present in the target nucleic acid.

47. The oligonucleotide of Claim 46 wherein the diagnostic nucleotide is the 3' terminal nucleotide (N) or N-1.

15 48. The oligonucleotide of Claim 46 further comprising a nondiagnostic nucleotide within about one to fifteen nucleotides from the diagnostic nucleotide.

49. The oligonucleotide of Claim 48 wherein the nondiagnostic nucleotide is within about one to five nucleotides from the diagnostic nucleotide.

20 50. The oligonucleotide of Claim 49 wherein the diagnostic and nondiagnostic nucleotides, respectively, are selected from the group consisting of N and N-3, N-1 and N-2, and N-2 and N-3.

51. The oligonucleotide of Claim 46 which hybridizes downstream from an amplification primer for the target nucleic acid.

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52. An oligonucleotide which is an amplification primer for an isothermal nucleic acid amplification reaction, the oligonucleotide comprising:

- 30 a) a 3' terminal nucleotide which is complementary to the target, and;
- b) about one to four nucleotides from the 3' terminal nucleotide, a diagnostic nucleotide for a single nucleotide polymorphism which may be present in a target to be amplified.

53. The oligonucleotide of Claim 52 wherein the diagnostic nucleotide is at N-1 or N-2.

35 54. The method of Claim 25 further comprising, prior to amplifying, displacing the hybridized detector primer from the target by extension of an upstream primer.

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